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Abstract: Background: Cystic fibrosis (CF) is the most common life-limiting inherited disease in Caucasian populations. While pathological changes can be seen in various organs, morbidity and mortality are mainly related to the respiratory tract, with patients suffering from chronic bronchopulmonary infections with characteristic pathogens including *Staphylococcus aureus*. Objectives: To date, there is only very limited data on the genetic and phenotypic characteristics of *S. aureus* in CF patients. Therefore, in our study, we characterized 58 *S. aureus* isolates collected from CF patients in Austria by spa typing, DNA microarray profiling, as well as antimicrobial susceptibility testing in order to determine common genomic and antimicrobial resistance features. The tested strain collection exhibited high genomic diversity. Results: The 58 isolates were assigned to 16 clonal complexes and 48 spa types and differed greatly regarding their virulence and resistance gene profiles. The predominant clonal complexes were MLST CC30 (22%), CC15 (16%), CC45 (14%), and CC5 (12%), complexes that are highly prevalent worldwide among *S. aureus* strains isolated from humans colonized or infected with *S. aureus*. DNA microarray profiles showed a wide variety of genes encoding antimicrobial resistance and virulence factors such as various leukocidins, haemolysins, enterotoxins, exfoliative toxins, toxic shock syndrome toxin, as well as genes involved in adhesion and immune evasion. Conclusions: While a large number of strains exhibited resistance to one or several antimicrobial agents, methicillin-resistant *S. aureus* was found at a low prevalence of 3% (n = 2) only. The two methicillin-resistant *S. aureus* isolates were assigned to CC152/t355 (SCCmecV) and CC5/t001 (SCCmecI). This is the first study to genetically characterize *S. aureus* isolates in CF patients in Austria

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Genetic and Phenotypic Characteristics of *Staphylococcus aureus* Isolates from Cystic Fibrosis Patients in Austria

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Key Words

Staphylococcus aureus · Cystic fibrosis · *spa* typing ·
Antimicrobial resistance · Virulence genes

Abstract

Background: Cystic fibrosis (CF) is the most common life-limiting inherited disease in Caucasian populations. While pathological changes can be seen in various organs, morbidity and mortality are mainly related to the respiratory tract, with patients suffering from chronic bronchopulmonary infections with characteristic pathogens including *Staphylococcus aureus*. **Objectives:** To date, there is only very limited data on the genetic and phenotypic characteristics of *S. aureus* in CF patients. Therefore, in our study, we characterized 58 *S. aureus* isolates collected from CF patients in Austria by *spa* typing, DNA microarray profiling, as well as antimicrobial susceptibility testing in order to determine common genomic and antimicrobial resistance features. The tested strain collection exhibited high genomic diversity. **Results:** The 58 isolates were assigned to 16 clonal complexes and 48 *spa* types and differed greatly regarding their virulence and resistance gene profiles. The predominant clonal complexes were MLST CC30 (22%), CC15 (16%), CC45 (14%), and CC5

(12%), complexes that are highly prevalent worldwide among *S. aureus* strains isolated from humans colonized or infected with *S. aureus*. DNA microarray profiles showed a wide variety of genes encoding antimicrobial resistance and virulence factors such as various leukocidins, haemolysins, enterotoxins, exfoliative toxins, toxic shock syndrome toxin, as well as genes involved in adhesion and immune evasion. **Conclusions:** While a large number of strains exhibited resistance to one or several antimicrobial agents, methicillin-resistant *S. aureus* was found at a low prevalence of 3% (n = 2) only. The two methicillin-resistant *S. aureus* isolates were assigned to CC152/t355 (SCCmecV) and CC5/t001 (SCCmecI). This is the first study to genetically characterize *S. aureus* isolates in CF patients in Austria.

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Introduction

Cystic fibrosis (CF) represents the most prevalent life-limiting inherited disease in Caucasian populations [1]. While all organs expressing the cystic fibrosis transmembrane conductance regulator (including secretory cells, airways, liver, pancreas, and the reproductive tract) can

be affected, the most severe pathological changes are usually seen in the respiratory tract [2]. Airway pathology is characterized by obstruction, inflammation, and severe recurrent and/or chronic bacterial infections. One of the most prevalent organisms that can be detected in CF patients is *Staphylococcus aureus*, which was reported to represent one of the first pathogens infecting the airways of CF patients and persisting for months or even years [3]. During the last decades, infections with both methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA) have increased considerably in CF patients in the United States. Prevalence of *S. aureus* in CF patients' respiratory cultures increased from 29% in 1992 to 69% in 2012, with the prevalence of MRSA increasing from 9% in 2002 to 25% in 2012 [4]. The same trend can be observed in European countries, whereas CF centres here report a lower rate of MSSA and MRSA in CF patients [5, 6]. Beside asymptomatic colonization of a patient, MSSA and MRSA can provoke a broad spectrum of infections, such as local infections up to endocarditis, pneumonia or sepsis. There are several publications reporting that MRSA-positive CF patients exhibit more complications, such as an increased hospitalization rate, an accelerated decline in lung function and an increased mortality rate [7, 8]. Effective treatment of both MSSA and MRSA infections represents a major challenge, as *S. aureus* exhibits a huge repertoire of different resistance and virulence factors [9].

To date, it is still poorly understood which virulence factors are crucial in the pathogenesis of *S. aureus* infections in CF patients, and there is only little information on genomic and antimicrobial features of these strains. Microbiologic laboratories offer a number of different molecular typing methods, such as *spa* typing or more recently microarray techniques, which allow a more detailed view on the epidemiological as well as the genetic background of the investigated strains. Therefore, in this study, 58 isolates collected from 51 CF patients in Austria were *spa* typed, and resistance and virulence gene profiles were investigated by DNA microarray analysis in order to gain new insights into the genomic and antimicrobial characteristics of *S. aureus* strains in CF patients.

Materials and Methods

Bacterial Strains

All *S. aureus* isolates included in this study were investigated at the CF Laboratory at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Austria. Samples originated from 51 CF patients and were collected over a

period of 1 year in the CF centre at the Respiratory and Allergic Disease Division, Department of Paediatrics and Adolescent Medicine, Medical University of Graz. A total of 23 (45%) patients were male and 28 (55%) female. The patients' age ranged from 1 to 41 years, with a median age of 16 years. Samples included sputa ($n = 46$), throat swabs ($n = 10$), and bronchoalveolar lavage fluids ($n = 2$).

Species Identification and Antimicrobial Susceptibility Testing

S. aureus isolates were identified using a VITEK 2 (bioMérieux, Marcy l'Etoile, France) or MALDI-TOF MS instrument (Axima™ Assurance, Shimadzu, Japan). Susceptibility testing was performed using both disk diffusion (Becton Dickinson, Heidelberg, Germany), according to the guidelines of the European Committee of Antimicrobial Susceptibility Testing, evaluating the susceptibility to penicillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg), and the VITEK 2 instrument (using the P580 card). Results were interpreted according to the guidelines of the European Committee of Antimicrobial Susceptibility Testing [10]. *S. aureus* with identical resistance phenotypes that had been isolated from the same patient were excluded from the study.

Molecular Characterization by *spa* Typing and DNA Microarray

spa typing is a widely used technique for subtyping *S. aureus* for epidemiological surveillance in hospital and community settings. The *spa* typing method is based on sequencing of the polymorphic X region of the protein A gene (*spa*), present in almost all strains of *S. aureus*. We extracted DNA and performed *spa* typing as described by Ruppitsch et al. [11]. StaphyType DNA microarray (Clondiag Chip Technologies, Jena, Germany) was used according to the manufacturer's instructions to assign isolates to clonal complexes and to generate virulence and resistance gene profiles. This microarray system detects the presence/absence of genes conferring resistance to antimicrobial agents, as well as genes encoding for various virulence factors such as haemolysins, leukocidins, enterotoxins, and exfoliative toxins. In order to visualize the diversity of DNA microarray patterns, as well as strain relatedness, hybridization patterns were analysed using the SplitsTree software (www.splitsree.org), designed to compute unrooted phylogenetic networks from molecular sequence data [12]. DNA microarray gene profiles were converted to 'sequence-like' strings of information as previously described [13].

Ethics Statement

No ethical clearance was necessary, as the strains were isolated for diagnostic purposes and the patients were informed and consented in writing to the anonymized use of the isolates for study purposes.

Results

As 6 samples harboured 2 ($n = 5$) or 3 ($n = 1$) *S. aureus* isolates with different resistance phenotypes, a total of 58 *S. aureus* isolates collected from 51 CF patients were included in the study. Among the investigated isolates, we frequently detected isolates resistant to one or more anti-

Table 1. Antimicrobial resistance phenotypes of 58 *S. aureus* isolates collected from CF patients in Austria

n (%)	Antimicrobial agents					
	OX	P	ERY	CLI	G	TM
22 (38)	–	X	–	–	–	–
14 (24)	–	X	X	(X) ^a	–	–
8 (14)	–	–	–	–	–	–
3 (5)	–	X	–	–	X	X
2 (3)	–	X	X	–	–	–
1 (2)	–	–	X	(X) ^a	–	–
1 (2)	–	X	X	–	–	–
1 (2)	–	X	–	–	–	X
1 (2)	–	X	X	–	X	–
1 (2)	–	X	X	(X) ^a	–	X
1 (2)	–	X	X	(X) ^a	X	X
1 (2)	X	X	X	X	–	–
1 (2)	–	X	X	X	X	X
1 (2)	X	X	X	(X) ^a	–	X
Total, n	2	49	21	2 (18) ^a	6	8
Total, %	3	84	41	3 (31) ^a	(10)	14

OX = Oxacillin; P = penicillin; ERY = erythromycin; CLI = clindamycin; G = gentamicin; TM = trimethoprim.

^a Inducible clindamycin resistance

biotic agents, including 2 (3%) MRSA strains (table 1). A high number of strains showed resistance to penicillin (84%), erythromycin (41%), trimethoprim (14%), and gentamicin (10%), and 31% of the isolates exhibited inducible clindamycin resistance. The 58 *S. aureus* isolates were assigned to 16 MLST clonal complexes and 48 different *spa* types (fig. 1). The most common clonal complexes were CC30 (22%), CC15 (16%), CC45 (14%), and CC5 (12%). While most *spa* types were unique, t002, t084, and t521 were found in 3 patients each, and t005, t9536, t021, and t335 were found in 2 patients each. The 2 MRSA isolates were assigned to CC152/t355 (SCCmecV) and CC5/t001 (SCCmecI).

The results of the DNA microarray showed an overall high genomic diversity among the *S. aureus* isolates, which is depicted in the SplitsTree (fig. 2). While *S. aureus* of the same clonal complex formed clusters, all isolates exhibited unique DNA microarray patterns and are thus representing different strains.

Regarding resistance genes (online suppl. table 1; see www.karger.com/doi/10.1159/000377707 for all online

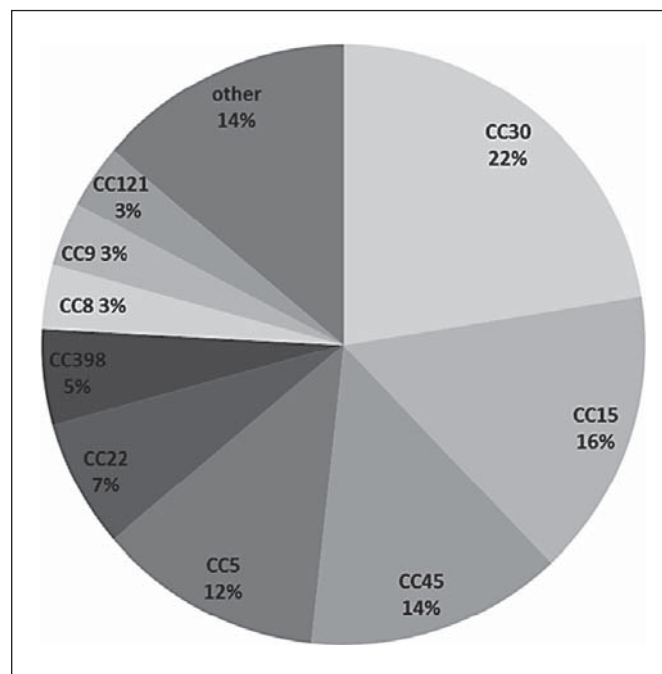


Fig. 1. Genotypes and assignment to clonal complexes of 58 *S. aureus* isolates from CF patients from Austria. Distribution: CC30 (13 isolates), CC15 (9 isolates), CC45 (8 isolates), CC5 (7 isolates), CC22 (4 isolates), CC398 (3 isolates), and CC8, CC9, and CC121 (2 isolates each). The unlabeled minor isolates were CC1, CC6, CC7, CC25, CC25/28, CC101, and CC152 with 1 isolate each, and there was 1 not assignable isolate.

suppl. material), 86% of the *S. aureus* isolates exhibited *blaZ/R/I*, involved in resistance to penicillin, and 3% harboured the *mecA* gene conferring resistance to methicillin. Genes involved in resistance to macrolides, lincosamides, and streptogramin were found in 11 (19%; *ermA*), 1 (2%; *ermB*), and 6 (10%; *ermC*) of the isolates. Concerning resistance to aminoglycosides, 11 isolates (19%) exhibited a positive result for *aacA-aphD* (involved in resistance to gentamicin and tobramycin), 3 isolates (5%) for *addD* (conferring resistance to tobramycin and neomycin), and 1 isolate (2%) for *aphA* (involved in resistance to kanamycin and neomycin). The resistance gene *dfrA* (conferring resistance to trimethoprim) was found in 5 isolates (9%). In none of the *S. aureus* isolates, resistance genes for other agents, including genes conferring resistance to vancomycin, teicoplanin, linezolid, or mupirocin, were found. Concerning the detected virulence genes, a wide variety of genes encoding superantigens, including toxic shock syndrome toxin, major enterotoxins, and exfoliate toxins, were detected, as well as various haemolysins, leukocidins, and adhesins (online suppl. table 2).

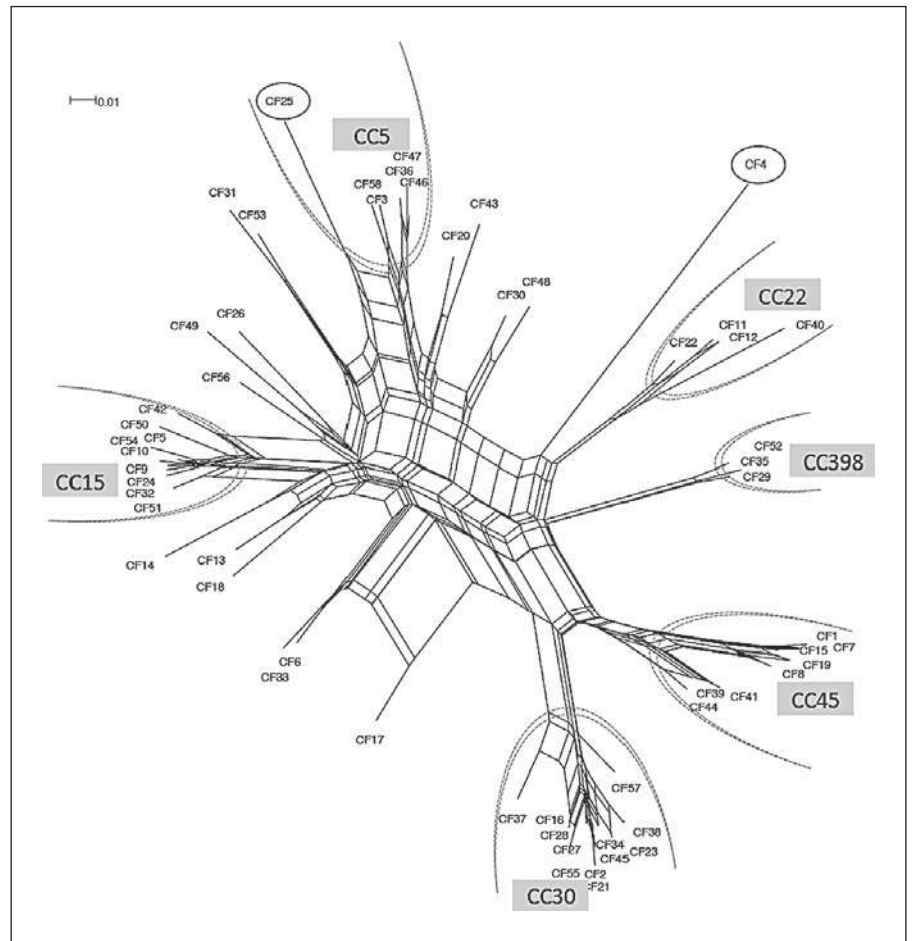


Fig. 2. SplitsTree depicting similarity of resistance and virulence gene profiles determined by DNA microarray for 58 *S. aureus* isolates from CF patients in Austria. Clonal complexes are indicated for clusters comprised of 3 or more isolates. The 2 MRSA isolates are marked by black circles.

The gene coding for the pore-forming exotoxin Pantone-Valentine leukocidin (PVL) could not be detected in any of the isolates. Assignment to *agr* types showed the following distribution: 27 isolates (47%) belonged to *agr* I, 18 (31%) to *agr* II, 15 (26%) to *agr* III, and 10 (17%) to *agr* IV. A comprehensive list of all microarray results, including genes encoding haemolysins, leukocidins, adhesins, as well as proteins involved in immune evasion, is provided in the supplementary material.

Discussion

Within the last years, several studies have reported a dramatic increase in MRSA strains isolated from CF patients, with MRSA rates of up to 25% [4]. While for many decades, MRSA was solely associated with health care-associated transmission and infections, so-called community-acquired MRSA (CA-MRSA) can now be found

both in the community but also in the hospital setting [5, 6]. It has been controversially discussed if the increase in CA-MRSA, which frequently carry PVL, represents a new threat to CF patients [14, 15]. PVL has been epidemiologically associated with severe skin and soft tissue infections, up to necrotizing pneumonia or severe sepsis [16].

In this study, MRSA could be detected in only 2 patients (3%), and none of the investigated strains was positive for PVL. A similar result was also reported by Campana et al. [14] who investigated MRSA isolates from 9 Italian CF centres. The MRSA isolate carrying SCCmecV was assigned to CC152/t355, which was recently detected among CA-MRSA in Austria [17, 18]. The MRSA isolate carrying SCCmecI was assigned to CC5/t001, which was reported in association with a hospital-acquired MRSA lineage (presumed Southern Germany clone) [19]. The overall low percentage of MRSA detected in our study is in accordance with previous reports describing constantly low local MRSA rates [17]. In addition, the CF centre

at which the samples were collected follows a strict hygiene regimen to avoid person-to-person transmission. This includes single rooms for inpatients, as well as a strict separation of outpatients with known colonization with *Pseudomonas* spp., *Burkholderia* spp., MRSA, and nontuberculous mycobacteria. It is conceivable that the fact that each *S. aureus* strain was found only once among CF patients in our study is at least partly due to this strict hygiene regimen.

We did not only detect MRSA but also a multitude of strains resistant to penicillin, erythromycin, clindamycin, gentamicin, and trimethoprim. Only 14% of the isolates in this study were susceptible to all tested antimicrobial agents. The high rate of resistant strains mirrors a high selective pressure due to the antibiotic regimens that were used to treat CF patients. In the local CF centre, prophylactic flucloxacillin to prevent infection with *S. aureus* or chronic maintenance therapy with oral antistaphylococcal antibiotics are not used, in accordance with current guidelines [20]. However, as in many other CF centres and in line with agreed treatment standards, maintenance therapy with azithromycin was used in a number of patients chronically infected with *Pseudomonas aeruginosa* due to the dual effect of macrolides on infection and inflammation, despite concerns regarding the induction of resistance. In addition, it is standard to recommend the use of amoxicillin/clavulanic acid or other antistaphylococcal antibiotics (e.g. flucloxacillin) for a minimum of 2 weeks in case of viral respiratory tract infections, and for at least 4 weeks with pulmonary exacerbations if *S. aureus* is suspected to be the cause of the exacerbation. When a patient with pulmonary exacerbation has to be admitted for intravenous antibiotic therapy and *S. aureus* is isolated, an antistaphylococcal antibiotic is added to the treatment regimen.

The 58 *S. aureus* strains investigated in this study were assigned to a wide variety of clonal complexes and *spa* types and exhibited pronounced diversity regarding resistance and virulence gene profiles. All clonal complexes detected in this study can be commonly found among *S. aureus* strains isolated from humans colonized or infected with *S. aureus* [13, 21, 22]. Furthermore, there is evidence that the main reservoir of the *S. aureus* isolates is not the clinical setting, due to the predominance of MLST CC30 and CC15, described as community-associated *S. aureus* [23].

There are reports that after the first acquisition of *S. aureus*, these isolates persist for a longer period. In a study by Hirschhausen et al. [24], long-term persistence of >5 years with the same individual and isogenic *S. aureus*

strain was detected. In vivo adaption processes could be observed, but the adaption processes seem to be very complex and might be influenced by individual host factors.

Some limitations of the study have to be addressed. While knowing the local antibiotic regimen, for this study, no detailed information on the antibiotic therapy of each individual CF patient or the clinical status at the day of sampling was retrieved. Beside that, this study was conducted in only a single CF centre in Austria and might be influenced by geographic or epidemiological factors. However, based on the fact that the same individual *S. aureus* strain might continuously colonize a CF patient, further studies could focus on the impact of these microbiological findings concerning the clinical status in these patients.

Conclusions

To conclude, this study provides for the first time information on the genomic background of *S. aureus* strains isolated from CF patients in Austria. In contrast to the high prevalence of MRSA reported among *S. aureus* isolates from CF patients in the US, only few MRSA isolates were detected (3%). The *S. aureus* isolates investigated in this study exhibited high genetic diversity with regard to clonal complexes, *spa* types, as well as resistance and virulence gene profiles. This indicates that acquisition of these strains more likely occurred in the community setting than by patient-to-patient transmission.

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